PDU Run P950310CF Final Report

September 26, 1995

1.0 Introduction

The primary purpose of this run was to test the continuous operation of the feed handling, pretreatment, and fermentation sections of the Process Development Unit (PDU). Corn fiber was the feedstock used for this run. The plan was to operate feed handling and pretreatment equipment continuously to produce a pretreated feed. This feed would then be continuously converted to ethanol in the four 9000-L fermentation vessels using the simultaneoussaccharification and fermentation (SSF) process. The fermentative organism was L1400 that was grown in the PDU seed train using the 20-L, 160-L, and 1450-L fermenters. Back-end unit operations (i.e., distillation, solid separation) were not used in this run.

As expected, not all of the equipment was able to operate for an extended period of time. A new pilot plant investigating new technologies would be expected to encounter difficult start-up and unknown operational problems. However, the run did identify these problems that could only be investigated with an extended run (e.g., reliability of the equipment and controls). Particularly important was integrating the unit operations of feedstock handling, pretreatment, and fermentation. This integration provided important training and operating experience with continuous operation of the plant and identified modifications needed for future runs.

1.1 Pilot Plant Configuration

This section provides a brief description of the PDU as background on the overall process. A simplified process flow diagram for the PDU is shown in Figure 1. The figure shows the overall flow path and equipment in the PDU. The process begins with feedstocks being loaded into a storage hopper (SH-120) and then continuously fed by a weigh belt (SA-150) to a mill (ND-110) by a pneumatic system. The niilled particles are separated from the air steam by a cyclone (FG-110) and fed to another weigh belt (SA-120). This belt controls feed rates to the rest of the plant. The feedstock is conveyed (SC-120) to a mixer (MX-250) and mixed with acid and water. The acidified biomass to fed to a high-temperature, high-pressure pretreatment reactor (MX-204) by a plug feeder (MX-270) that creates a impervious biomass plug. Temperature, acid concentration, and residence time are controlled in the reactor to achieve adequate pretreatment. The pretreated material is then cooled by flashing to the flash tank (MX-205). This material is then puniped (P-205) to the first 9000-L fermenter (V-455A), or alternatively, it can be pumped to a hold tank (V-210A) for storage.

In the first 9000-L fermenter, pretreated biomass is combined with inoculum, cellulase enzyme (from V-321), and corn steep liquor (from V-420). The microorganism is started in a shake flask and successively transferred to the 20-L fermenter. 160-L fernienters (V-445A/B), 1450-L fermenters (V-450A/B), and finally to the seed hold tanks (V-465A/B) to await addition to the first 9000-L fermenter. Once the first fermenter is filled, fermentation broth is pumped to the other 9000-L fermenters (V-455B/C/D) in the train. Each fernienter receives continuous feed and its level is controlled to maintain a preselected residence time. Exhaust gas from the fermenters is sent to a scrubber (T-460) to remove volatile organics and odors. The beer well (V-510) receives and holds spent fermentation broth from the last fermenter (V-455D).

Femientation broth in the beer well can be pumped to either the neutralization tank (V-602) for pH adjustment and then disposal, or pumped to the distillation column (T-501) for removal of the ethanol. Partially purified ethanol from the column is condensed and sent to the ethanol storage tank (V-506). The stream from the bottom of the column is cooled (in E-506) and sent to a feed tank (V-601). This material is then pumped to

the centrifuge (FF-610) to remove the remaining solids, which drops into the cake tank (V-611). The solids are then sent to disposal through V-602. The liquid fraction from the centrifuge is collected in the centrate tank (V-610); this liquid can then be sent to disposal (through V-611 and V-602) or to the sterilization tank (V-256). Sterilized liquid is held in a feed tank (V-257) and can then be used as make-up water for the process.

2.0 Pilot Plant Operations

Operation of the plant began on March 12 and continued until March 27 utilizing feed handling, pretreatment, and fermentation equipment. Operating conditions evolved during the run as process performance information became available and necessitated changes to the preselected variables. These changes are discussed in the next section. Additionally, a run history and significant operational notes are presented.

2.1 Procedures and Operating Conditions

2.1.1 Feed Handling/Pretreatment Operating Conditions

Corn fiber was selected because it is a relatively easy-to-handle feedstock and of interest for CRADA work. Corn fiber was obtained from a local corn wet-milling facility (Golden Technologies, Inc., Jolmstown, CO). It was conveyed by the pneumatic system through the mill and cyclone to remove heavy contaminates. Table 1 shows the operating conditions for feedstock handling and pretreatment sections of the PDU. The plant was initially operated using the first set of parameters for pretreatment. After two days, the conditions were changed to the second set of parameters (less severe conditions) to reduce the amount of fermentation inhibitors produced during pretreatment, which may have led to lack of ethanol production in the 9000-L fermenters (to be discussed later in this report).

Typically, there were cyclic variations in pretreatment conditions around the setpoints values on the order of ± 4°C for temperature, ± 2 min for residence time, and ± 0.05% for acid concentration. Some variability is unavoidable, however, attempts will be made in the future to improve or at least characterize the performance of these control loops. Better tuning and/or hardware changes will be worked on in the future.

Table 1. Feedstock Handling and Pretreatment Operating Conditions

Feedstock Handling:	Feedstock Flow Rate (dry kg/h)	38
	Feedstock Moisture Content (%)	60
Pretreatment:	Temperature (°C)	170, 160
	Residence Time (min)	15, 10
	Acid Concentration (%)[a]	0.7, 0.7
	Solids Concentration (%)[b]	25, 25
Flash Tank:	Solids Concentration (%)[b]	25, 15

[a] acid concentration in the liquid phase
[b] determined as dry solids divided total mass (solids + liquids)

2.1.2 Fermentation Operating Conditions

Operating conditions for the seed train are shown in Table 2. L1400 was grown by successive transfers from shake flask to the 20-L, 160-L, and 1450-L fermenters, respectively. There was no pH control in the shake flask. pH was controlled with 3.0 molar NaOH in the 20-L and 160-L fermenters and with 50% NaOH in the 1450-L fermenter. Inoculum from the 1450-L fermenter was transferred to the seed hold tanks to await addition to first 9000-L fermenter. The seed tanks were agitated at 50 rpm, maintained at a gauge pressure of 0.33 bar, and cooled with circulating cooling water.

Table 2. Fermentation Operating Conditions

Operating Condition	Flask #1	Flask #2	20-L	160-L	1450-L	9000-L
Temperature ("C)	30	30	30	30	30	30
Agitation (rpm)	150[a]	150[a]	150	100	75	50
pH	5.0	5.0	5.0	5.0	5.0	5.0
Pressure (bar)			0.33	0.33	0.33	0.33
Airflow (vvm)		-	0.5	0.5	0.25	0.03[b]
Residence Time (h)	8[c]	8[c]	8[c]	12[d]	12[d]	24[d]
Media:						
Glucose (%)	2	2	2	2	2	
Peptone (%)	2					
Yeast Extract (%)	1					
CSL (%)		1	1	1	1	1
Antifoam (mL/L)			0.5	0.5	0.5	
Enzyme (IU/g cellulose)						25[e]

[a] laboratory shaker agitation
[b] air added to maintain a positive pressure in vessels
[c] typical incubation times
[d] per 9000-L vessel
[e] substrate was pretreated corn fiber

Fermentation conditions in the 9000-L fermenters are also presented in Table 2. Corn steep liquor (CSL) and enzyme additions were only made to the first 9000-L fermenter. A 10% (v/v) inoculum from the seed hold tanks was also added to the first 9000-L fermenter. pH was controlled using 50% NaOH. Level was maually controlled in each 9000-L fermenter to maintain a residence time of 24 h.

2.2 Run History

A tinie line for this run is shown in Figure 2 for operation of the pretreatment reactor, seed train fermenters (20-L, 160-L and 1450-L fermenters), seed hold tanks, and the 9000-L fernienters. The pretreatment reactor was operated from March 13 to March 15 and then shut down because of a mechanical problem (see section 2.3.1). The reactor was restarted later in the day and operated for 1 day before being shut down for another

mechanical problem. These problems were fixed and the reactor was restarted on March 22 and operated smoothly for 54 h until the reactor was shutdown at the scheduled end of the run on March 24.

Seed train fermenters were started up as shown in Figure 2 and generally fed the next fermenter in the train. The 20-L fermenter was operated as needed to maintain a viable seed supply in case one of the larger fermenters became contaminated. The 1450-L fermenter was successfully operated in the draw and fill mode to provide inoculum for SSF. Fill and draw means that the fermenter is filled with fresh media while leaving approximately 10% of the previous ferrnenter contents to provide inoculurn for cell growth. This avoids operation of the smaller seed fermenters. Operation of the first 9000-L fermenter began on March 13 and continued until March 27. When each of the 9000-L fermenters was filled, transfers then began to the next 9000-L fermenter in the continuous train (V-455A to V-455B to V-455C to V-455D). The first fermenter received pretreated corn fiber until March 15, then both the first two fermenters were dumped (reasons are discussed in section 2.3.2). Pretreated feed addition to the first 9000-L fermenter began again and continued until March 16, when the pretreatment reactor was again shut down. The 9000-L fermenters were kept operating by glucose additions until pretreated feed was again started to the first fermenter on March 22, at which time the third and fourth fermenters could begin operation.

2.3 Operational Notes

The following is a list of significant operational notes and problems that occurred during this run.

2.3.1 Feed Handling/Pretreatment

- When the pneumatic system is used, the feed rate measurement is taken from the second downstream weigh belt ($A-120) and used for ratio control of other feed additions (e.g., acid and water). The measurement from this weigh belt is more variable than the upstream weigh belt (SA-150) measurement because of surging of the feed through the cyclone and airlocks. Future operation should avoid using the pneumatic system by screening the feedstock to remove contaminants.

- The pretreatment reactor was shut down from 9:00 am on March 15 to 10:00 pm on March 15 because the agitator on the bottom of the reactor was incorrectly installed without a key on the shaft.

- The pretreatment reactor was shut down 3t 9:00 pm on March 16 because of an unidentified squeak coming from bottom agitator. The equipment was taken apart and possible causes were determined to be an overtighten packing, poor or inadequate lubricant flow, or pretreated material seeping into the bushing. The packing was replaced and modifications were made to the lubrication system.

- **Initially,** pretreatment temperature was assumed to be indicated by the temperature probe (RTD) in the side wall of the reactor. After the head-space temperature probe (in top of reactor) was fixed on March 15, it was noticed that there was a large difference between these two probes that should have been reading the same (the side-wall probe read nearly 25°C lower than the head-space probe). The location of the side-wall probe might not have allowed adequate steam penetration and thus caused an erroneous reading. Because of this concern, temperature control of the pretreatment reactor was switched to the head-space probe at 6:00 am on March 16.

- Liquid and some solids are continually squeezed out of the wetted corn fiber by the plug flow feeder (MX-270). This material is collected in a small reservoir and pumped into the pretreatment reactor. There were continual plugging problems in the reservoir because of the large solids loading in the squeezed material. Modifications will be done to the system to decrease the solids loading and

increase the ability of the reservoir and pump to handle **solids.**

2.3.2 Fermentation

- Manual additions of CSL, enzyme, and inoculum were made because of problems with the addition valves to the first 9000-L fermenter.

- It was extremely difficult to **mix** the 25% (solids concentration) corn fiber slurry in the first 9000-L fermenter. The lack of mixing produced erroneous pH and temperature readings in this vessel (actual readings were 8.3 and *77°C* compared to 5.0 and 30°C before the probes were cleared) and these conditions may have inactivated previous enzyme and inoculum additions. Additional media was added and first fermenter was reinoculated at 11:00 pm on March 14.

- The first two 9000-L fermenters were dumped on March 15 because of **lack** of cell growth and ethanol production. This may have been caused by the incorrect temperature reading in the pretreatment reactor (as discuss above). The higher temperature produced more severe pretreatment conditions and thus high levels of components known to inhibit fermentation (e.g., acetic acid, furfural).

- Glucose was added to the first two 9000-L fermenter on March **17** after the pretreatment reactor was shut down. This was done to maintain viable yeast cells until pretreated corn fiber could again be added to the fermenters.

- Plugging occurred in the process lines between the 9000-L fernenters when the pumps at the bottom of the fermenters were turned off. These pumps were turned off when it was necessary to briefly shut off water to the seals to change out the sterile water filter.

- Plugging occurred in the caustic (NaOH) addition lines to the fermenters used for pH control. These lines were unplugged during the run and cleaning after this run should solve the problem in the future.

- Autoniatic level control in the 9000-L fermenters was not possible because of load cell transmitter and calibration problems at the control system that have not been resolved with the manufacturer. Level was monitored locally and maintain constant by routine adjustment of the speed of each fermenter's pump.

- The 9000-L fermenters required 150 L/min of makeup air to maintain a 0.33 bar gauge pressure. It should be possible to maintain pressure in the fermenters without makeup air addition simply from carbon dioxide production during the fernlentation process. The pressure control valves will be examined **and** fixed to reduce or eliminate the use of makeup air.

3.0 Key Results

The following sections present key results for pretreatment and the fernlentation processes.

3.1 Pretreatment

Figure **3** shows data from March 22 to March 24 (where March 22 at 06:00 is defined as time zero) on the acid concentration of corn fiber coming out of the mixer (MX-250) before being fed to the pretreatment reactor.

The acid concentration of a small sample of corn fiber exiting the mixer was determined by titration. The purpose of the mixer is to thoroughly blend water and acid into the feedstock. Since all the feed flow rates to the mixer are relatively constant, this measurement is an indication of the uniformity of feed after the mixing process. The results show a rather wide variation from 0.4% to 0.8%, which would indicate that the mixer does not uniformly mix the acid and water into the feedstock. This is not unexpected since, the residence time in the **mixer** is only several minutes. This may not be sufficient to get complete absorption of liquid into a biomass feedstock. It is not clear how serious this problem is because the acid solution is squeezed out of the feed and then added back to the feed in the hydrolyzer, which is likely to create a more uniform acid concentration and moisture level.

Figure **4** shows the concentration of monomeric sugars (glucose and xylose) and inhibitors (acetic acid and furfural) in the pretreated liquor stream **from** March 22 to March **24.** Relatively stable concentrations occurred throughout the 48 hour period, indicating stable operation of the pretreatment reactor.

During steady operation of the pretreatment reactor from March 22 to March 24, two set of samples (corn fiber, pretreated corn fiber, and flash vapor) were taken and analyzed to determine the pretreatment yields and material balance closure. These samples were taken from the exit of the flash tank that was operating with a 15% solids concentration. The results are shown in Tables 3 and **4.** The analytical data were produced by the PDU chemical analysis team and all results are based on total available sugars. The carbon balance closure for each sample was 102% and 95%, respectively, which is very good considering the number of data (e.g., multiple flow rate readings) and analysis results (e.g., liquor and solids compositions, moisture levels, etc.) required to calculate these balances. Although, the pretreatment conditions for both of these runs were nearly identical as shown on the tables (165°C, 10 min residence time, **and** 0.72% acid), there are significant differences in the glucose (47.4%, **52.7%)** and xylose (67.5%, 58.6%) yields. These yields are the fraction of total available sugars converted to monomeric and oligomeric sugars. The yield differences can probably be attributed to difficulties in accurately controlling residence time. Additionally, the relatively low xylose yields and large fraction of unconverted xylan indicates that the pretreatment conditions were not very severe. The fraction of the total sugars in the pretreated feed that were monomers were only 33% and 20% for xylose and glucose, respectively (data not shown in the table).

Table 5 shows a comparison of the composition of raw, pretreated, and SSF residue corn fiber by both the PDU analytical staff and staff from the Chemical and Analysis Testing (CAT) **Task.** For the most part, the results are comparable except for significant differences in values for galactose, mannose, acid soluble lignin, and starch. Because these difference are on minor constituents of the biomass and **are** consistent between raw and pretreated biomass, there is little effect on the pretreatment material balances.

3.2 Fermentations

Cell **and** ethanol yields on glucose in the seed fermentation train are shown in Table 6. These results are very uniform between the three fermentation vessels and are similar to previous data obtained in the seed train. This uniformity has not been seen in previous runs. When rebatching the **1450-L** fermenter using the fill and draw mode, holding the remaining broth in the bottom of the tank for 2 days produced a 24 h **growth** lag. The vessels will be turned around more often in the future to avoid holding fermentation broth for long periods of time.

Figure **5** shows some of the liquid component concentrations (monomeric glucose and xylose, acetic acid, and ethanol) in the first 9000-L fermenter over the course of this run (where March 14 at 20:00 **is** defined as time zero). Initially glucose is high and ethanol is low because there were no viable yeast **cells** during the early **part** of the **run.** The fermenter contents were then dumped after about 24 hours. The fermenter was refilled with pretreated corn fiber and reinoculated at about 70 hours, at which time glucose is consumed and ethanol

Table 3. PDU Pretreatment Material Balance

Run #: P95031OCF
Date: 3/22/95
Time: 20:30

Run Conditions:				
Hydrolyzer Temp (C) :	164	Flash Tank Temp. (C):	96
Hydrolyzer Residence Time (min) :	10		
Hydrolyzer Add Concentration (%):	0.74		

Input Data:

Feed Row Rate (SA-150,kg/h):	83.5	Feed Solids Concentration (%):	41.8
Water to Pug Mill (FT-250-1, kg/h):	24.4	V-201 Acid Concentration (%):	8.5
Acid Flow Rate (FT-201-1, kg/h):	10.8	Lime Concentration (%):	25.0
Steam to Hydrolyzer (FT-204-1, kg/h):	39.5		
Lime To Rash Tank (FT-201-3 kg/h):	6.5	Hydrolyzate Insoluble Solids (%):	6.22
Water to Rash Tank (FT-205-1, kg/h):	86.9		
Flash Vapor (kg/h):	17.1		

Carbon Balance: Pretreatment

	Unpretreated		Pretreated										
			In Solids			In Liquid			In Flash			Total	
Component	Dry Feed (% dry weight)	Carbon in (C-mole/h)	(% dry weight)	(C-mole/h)	(% C in Feed)	(g/L)	(C-mole/h)	(% C in Feed)	(g/L)	(C-mole/h)	(% C in Feed)	(C-mole/h)	(% C in Feed)
Glucose	33.8	392.895	40.7	196.346	50.0	25.6	186.203	47.4				382.549	97.4
Mannose	3	34.872	5	24.121	69.2	4.6	33.458	95.9				57.579	T65.1
Galactose	7.24	84.159	7.7	37.146	44.1	5.61	40.805	40.5				77.951	92.6
Xylose	22.71	263.983	15.8	76.223	28.9	24.5	178.202	67.5				264.653	100.3
Arabinose	14.68	170.642,	5.6	27,016	15.8	15.7	114.195	64.9				141.210	82.8
Acetic Acid						1.3	9.456		0.4	0.2		9,683	
Formic Acid						0	0.000						
Lactic Acid						1.9	13.820					13.820	
Lignin	14.4	240.202	28	193.838	80.7	6.67	69.619	29.0				263.457	109.7
Furfural						0.9	10.228	2.4	15.9	14.1	3.3		
HMF						0	0.000	0.0					
Total	86.9	1186.753	94.9	554.689	46.7		655.985	55.3		14.4	1.2	1210.902	

Ignores protein, Starchis included in glucose number

C - RECOVERY: 102.03%

Table 4. PDU Pretreatment Material Balance

Run #: P950310CF
Date: *3/23/95*
Time: 18:30

Run Conditions:				
Hydrolyzer Temp (C) :	165	Flash Tank Temp. (C):	97
Hydrolyzer Residence Time (min) :	10		
Hydrolyzer Acid Concentration (%):	0.73		

Input Data:

Feed Row Rate (SA-150,kg/h):	81.3	Feed Solids Concentration (%):	44.9
Water to Pug Mill (FT-250-1, kg/h):	*29.6*	V-201 Acid Concentratton (%):	6.5
Acid Flow Rate (FT-201-1, kg/h):	10.8	Lime Concentration (%):	25.0
Steam to Hydrolyzer (FT-204-1, kg/h):	40.9		
Lime to Flash Tank (FT-201-3 kg/h):	6.2	Hydrolyzate Insoluble Solids (%):	6.03
Water to Flash Tank (FT-205-1, kg/h):	81.9		
Flash Vapor (kg/h):	17.5		

Carbon Balance: Pretreatment

	Unpretreated		Pretreated											
			In Solids			In Liquid			In Flash			Total		
Component	Dry Feed (% dry weight)	Carbon In (C-mole/h)	(% dry weight)	(C-mole/h)	(% C in Feed)	(g/L)	(C-mole/h)	(% C in Feed)	(g/L)	(C-mole/h)	(% C in Feed)	(C-mole/h)	(% C in Feed)
Glucose	33.8	410.913	40.2	187.004	45.5	*29.9*	216.754	52.7				403.758	*98.3*
Mannose	3	36.472	4.8	22.329	61.2	5.4	39.146	107.3				61.475	168.6
Galactose	*7.24*	88.018	7.4	34.424	*39.1*	5.47	39.654	45.1				74.077	*84.2*
Xylose	22.71	276,090	15.4	71.638	25.9	22.3	161.659	58.6				240.094	87.0
Arabinose	14.68	178.468	5.2	24.190	13.6	15.2	110.189	'61.7				134.379	75.3
Acetic Acid						1.1	7.474		0.5	0.3		*8.266*	
formic Acid						0	0.000						
Lactic Acid						1.9	13.774					73.774	
Lignin	14.84	258.894	26.1	174.228	67.3	7.24	75.316	29.1				249.545	96.4
Furfural						0.6	6.796	1.5	16.9	15.4	3.4		
HMF						0	0.000	*0.0*					
Total	*87.4*	1248.855	*91.4*	*513.812*	*41.1*		*671.263*	53.8		*15.7*	*1.3*	*1185.366*	

Ignores protein.Starch is included in glucose number

C- RECOVERY: 94.92%

concentration increases. **Also** at this point, the pretreatment reactor was shut down because of a mechanical problem. The culture was then sustained on exogenous glucose additions. The ethanol concentration drops when pretreated corn fiber is again added to the fermenter at about 180 hours. This occurs because glucose concentration in the fermenter with pretreated corn fiber was lower than the glucose concentrations used to sustain the culture.

Table **5.** Analysis" of raw, pretreated, and SSF residue corn fiber **by** PDU and CAT analytical teams.

Sample	G	X	GA	**A**	M	LKL	LAS	Ash	ST[b]
Raw Corn Fiber									
PDU	33.8	22.7	7.2	14.7	3.0	**7.6**	7.2	1.1	16.0
CAT	33.4	23.7	3.9	**15.5**	0.1	6.5	3.4	1.0	15.7
Pretreated Fiber (3/22, 20:30)									
PDU	40.7	**15.8**	7.7	5.6	5.1	20.6	7.4	2.2	6.8
CAT	42.6	13.0	1.9	4.3	0.8	21.4	3.5	1.2	2.5
Pretreated Fiber (3/23, 18:30)									
PDU	40.2	15.4	7.4	5.2	4.8	18.8	7.3	1.9	7.2
CAT	41.4	13.9	2.1	5.0	0.3	19.9	1.0	1.0	3.3
SSF Residue									
PDU	9.5	6.7	6.9	3.3	9.0	35.4	7.3		3.4
CAT	11.3	3.8	0.6	1.3	3.6	45.0	6.8	2.6	0.5

G: Glucose
X: Xylose
GA: Galactose
A: Arabinose
M: Mannose
LKL: Klason Lignin
LAS: Acid Soluble Lignin
ST: Starch

[a] Based on total available sugars
[b] Since starch is a polymer of glucose, it is also included in the glucose number. This difference is cellulose.

Figure 6 shows ethanol concentration in the four 9000-L fermenters over the course of this run. High ethanol concentrations were achieved in the middle part of the run (96 to 180 h) because of glucose additions. With addition of pretreated feed began at 180 hours, ethanol concentrations began to drop because of the lower glucose content in the feed, but appeared to stabilize at successively higher ethanol concentrations in each fermenter down the fermentation train. This type of behavior would be expected because of continuing conversion occurring in each successive fernienter.

Table 6. Seed Train Cell and Ethanol Yields on Glucose

Fermenter	Cell Yield (g/g)	Ethanol Yield (g/g)
20-L	0.181	0.352
	0.116	**0.341**
	0.098	0.319
	0.085	**0.336**
160-L	0.078	0.334
	0.060	0.376
1450-L	0.168	0.330
	0.112	0.287

4.0 Other Issues

The following were other important issues that were addressed during this run.

4.1 Evaluation of Shift Work

Two different shift schedules were utilized during this run. An 8 h/shift, **5** day rotation with 2 days off was used for the pretreatment crews while a 12 h/shift, **4** day maximum rotation was implemented for the fermentation crews. For the 13 day run, most operators remained on the same shift.

Feedback from the fermentation operators indicated that for most of them, a **4** day rotation was too long. Many operators felt that they were not at their best **by** the fourth shift, and after having four days off, it took longer to come **up** to speed when they returned to work. The 12 hour shifts were acceptable to the majority of the operators because it allowed for more time off at a stretch. The next run will utilize a 3 day maximum rotation with 12 hour shifts **for** all of the operators except those operating the pretreatment system; they will rotate through an 8 hour shift.

4.2 Mass Spectrometer (MS) Operation

The MS **was** down for most of the **run** because a board in the electronics rack failed. Fisons Instruments, the MS manufacturer, was contacted as soon as the problem was identified and they sent a new board. After installation of the new board and configuration of the **trips** (trips are high pressure alarm states that will terminate the current run should a leak **in** the system arise), it was discovered that a glitch had developed in the sofhvare that required reloading of the software and subsequent reconfiguration. Following the completion of these tasks, the unit appeared to run well. Problenis of this type will continue to occur as the MS is **a** relatively delicate instrument especially when inconsistent power is supplied to the unit.

Several complaints were received from control room occupants while the unit was analyzing fernienter gases because of a strong fermentation odor that filled the room. This problem was solved by installation of a small exhaust hood over the MS sampler.

Finally, there was a communication problem between the MS and the data acquisition and control system that was fixed by a Fisons representative shortly after completion of the run.

4.3 Contamination Issues

No contamination was detected in any of the *seed* fermenters or seed holding tanks. Both the streak plate method on YPD (Yeast Extract, Peptone, Dextrose) agar plates and inoculated liquid YPD medium containing nystatin were used to select for bacterial contaminants.

Contamination in the first 9000-L fermenter was first detected on March 18 using the liquid YPD media. Streak plates for the same date were clean and microscopic examination was inconclusive. However, contaminates did appear at low levels on YPD plates on samples taken from the first two 9000-L fermenters on March 21. The amount of contaminants increased considerably from March 23 to March *25* as seen on YPD plates. The contaminant was a small Gram-positive rod, possibly a bacillus species. This type of organism has been seen before in bench scale SSF's, including a SSF on a sample of pretreated corn fiber from this run.

There are several possible sources of contamination: the CSL, the unfiltered enzyme, the corn fiber, or mechanical seal problems. Both CSL and enzyme have been carefully checked and no contamination was found in either the CSL feed tank or the enzyme feed tank. The pretreated corn fiber is a potential source of contaminants because of the high microbial load in the raw corn fiber before pretreatment. Although pretreatment conditions are severe enough to kill microorganisms (160°C, 10 min), there may be some clumped material not subjected to the harsh conditions or because of the high initial microbial load, some microorganisms or spores may survive. If this occurs, contamination would eventually appear in a long enough run. Methods to determine microbial loads in raw substrates have been identified in the literature. A bacterial typing kit has been ordered so that contaminants can be identified in the future.

5.0 Summary

As expected for the first major extended run of the PDU, there were many mechanical and operational difficulties. The pretreatment reactor was shut down twice for mechanical problems. When the seal and agitator problems on the reactor were fixed, the reactor operated well during the last two days of the run, however, we still need to verify longer term operation. There were also plugging problems in the squeezate reservoir and some control problems in the pretreatment system that will be worked on before the next run. Sensor and analytical data taken from the pretreatment system also showed promising mass balance closure.

The fermentation seed train for this run operated well and produced uncontaminated inoculum for the main femienters. The 9000-L fermenter operation showed that repairs and some operational changes are necessary to make the systeni functional and more reliable. For example, automatic feed addition of CSL, enzyme, and inoculum did not work because of valve and flowmeter problems. Automatic level control did not operate because of load cell problems. The pressure controls valves on the 9000-L fermenters did not provide a good seal. All these equipnient issues will be worked on before the next run.

There were operational problems in the 9000-L fermenters such as poor mixing during startup of the first fernienter that led to incorrect pH and temperature readings. This can be solved in the future by initially adding dilution water until enzyme hydrolysis thins the broth. Conditions that led to plugging problems in the fernienter feed lines will also be avoided in the future. Finally, because of the intermittent operation of the pretreatment reactor, it was not possible to calculate meaningful yields or material balances on the fermentation systeni.

6.0 Future Work

The next run is scheduled for April and will be an extended run using the Amoco Pretreatment Reactor. Problems that were identified during this run with fermentation and pretreatment equipment will be fixed. Of primary concern are the CSL, enzyme, and inoculum addition valves. With these measurements, it should also be possible to obtain material balance information around the fermentation system.

7.0 Acknowledgments

The following PDU staff members contributed to the successful operation of the plant during this run: Brian Boynton, John Brigham, Nancy Combs, James Dickow, Roger Duwe, Jody Farmer, James Hora, Kelly Ibsen, Ed Jennings, James Johnson, Tim Johnston, Will Keutzer, Quang Nguyen, Robert O'Conner, Tim Plumrner, Mark Ruth, Dan Schell, Ralph Smith, Ryan Stoner, Ian Thompson, Susan Toon, and Mel Tucker. Christos Hatzis supplied the original material balance spreadsheet that was subsequently modified for use with PDU data. This report was put together with written contributions from Brian Boynton, Nancy Combs, Jody Farmer, Kelly Ibsen, and Dan Schell.

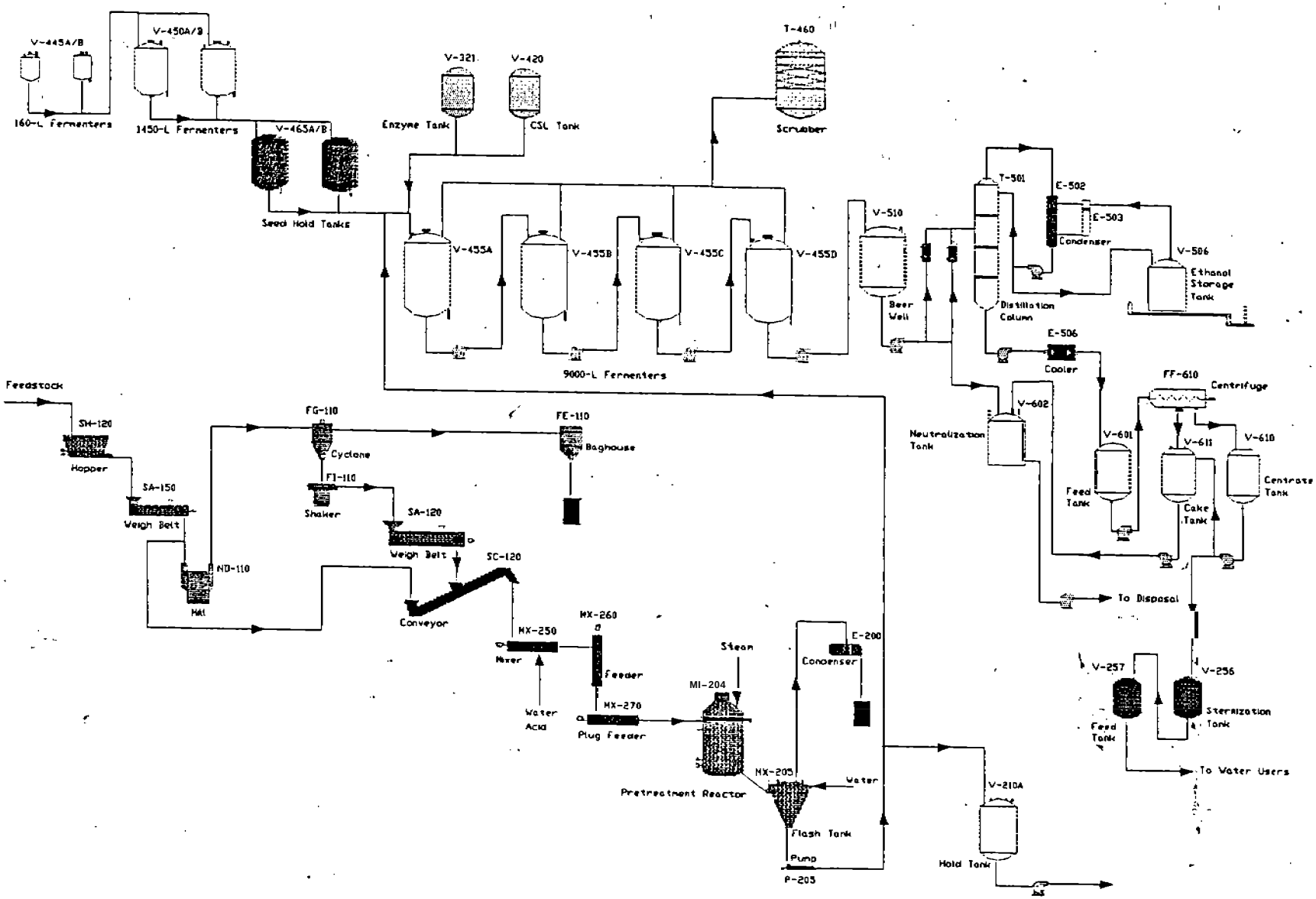


Figure 1. PDU Process Flow Diagram

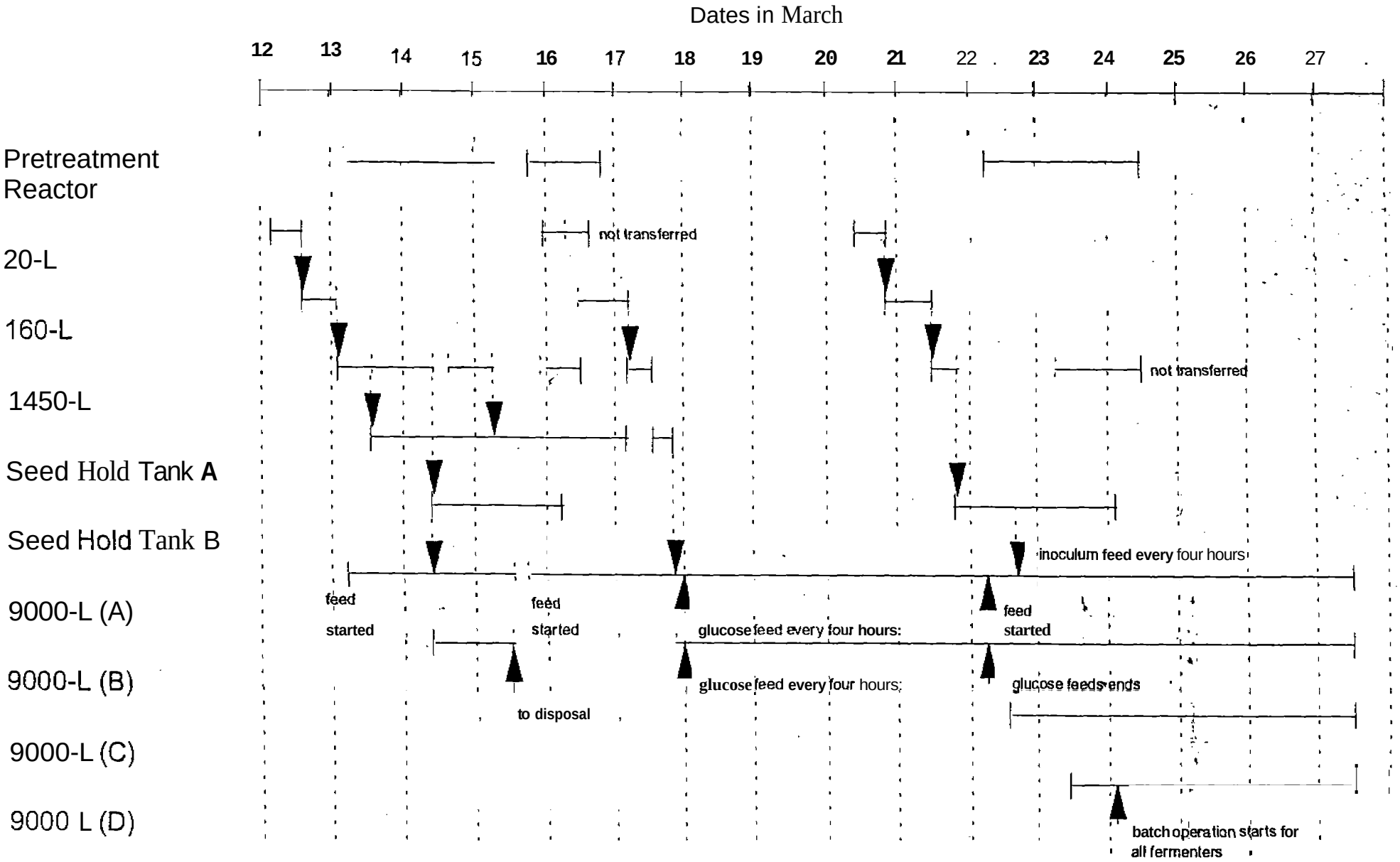


Figure 2. PDU Run History

Figure 3. Acid Concentration of Corn Fiber from the Pug Mill

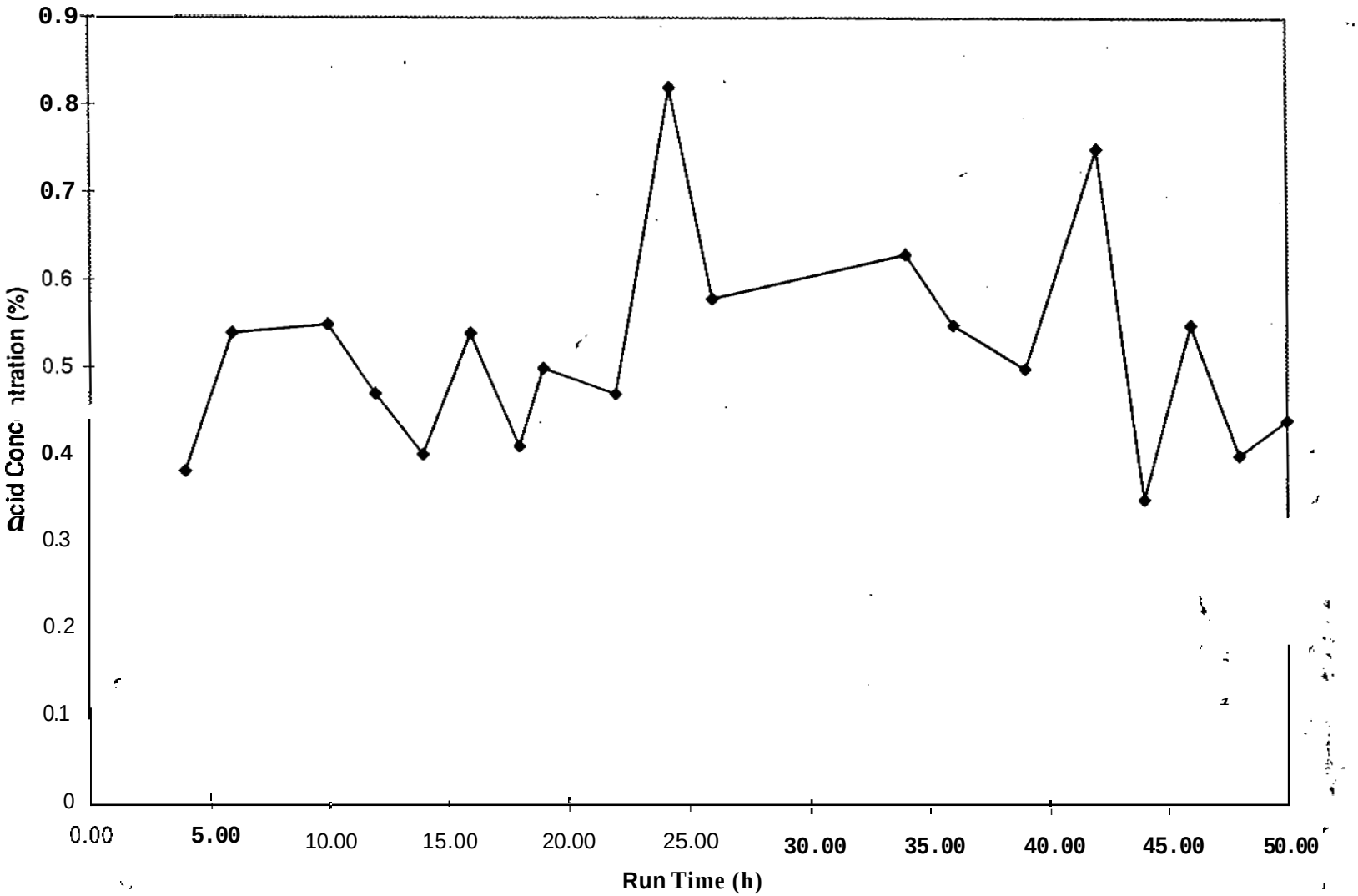


Figure 4. Corn Fiber Hydrolyzate Composition From Flash Tank
163 C, 0.7% acid, 10 min residence time, 15% solids

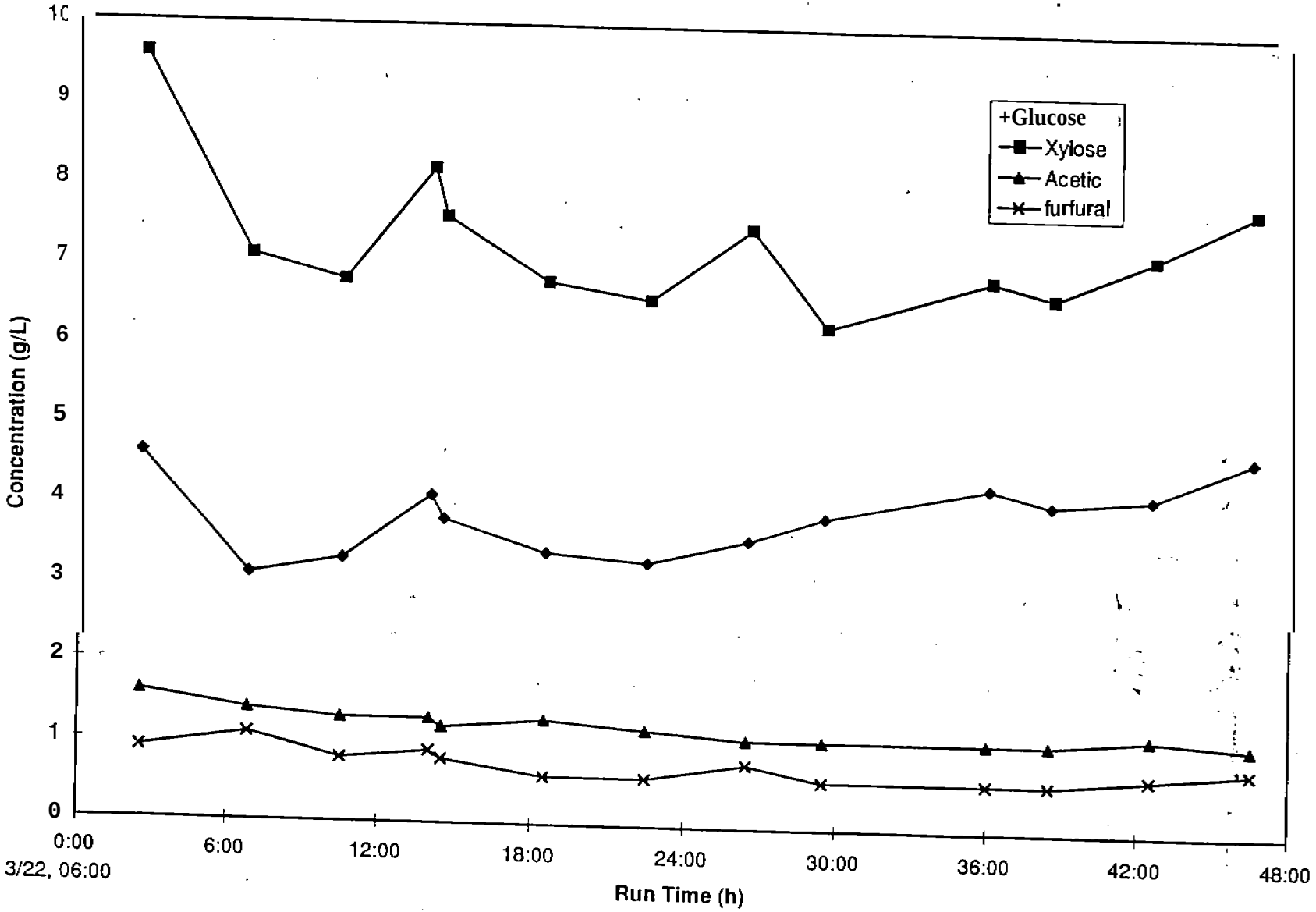


Figure 5. Component Concentrations in the First 9000-L Fermenter

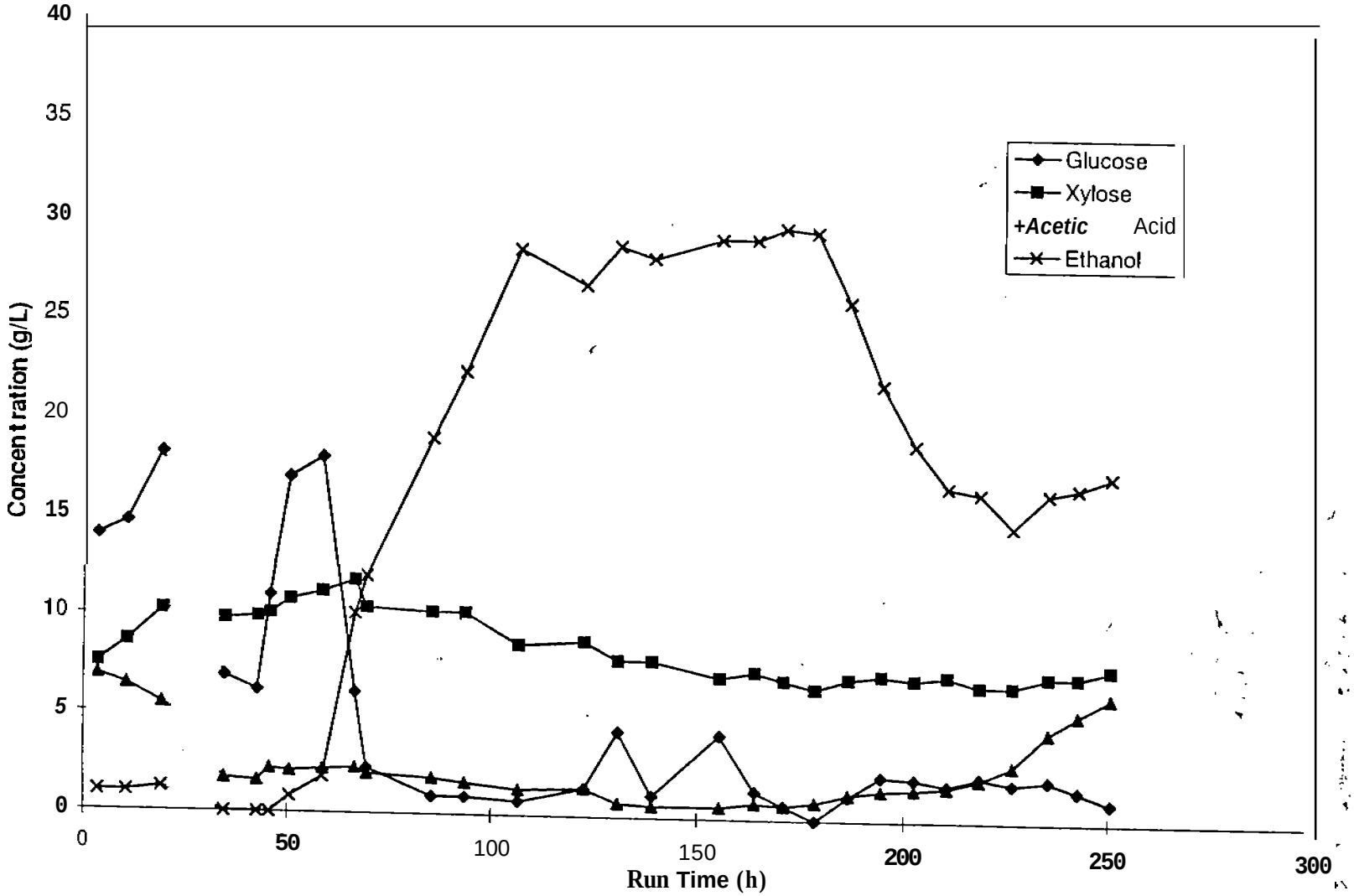
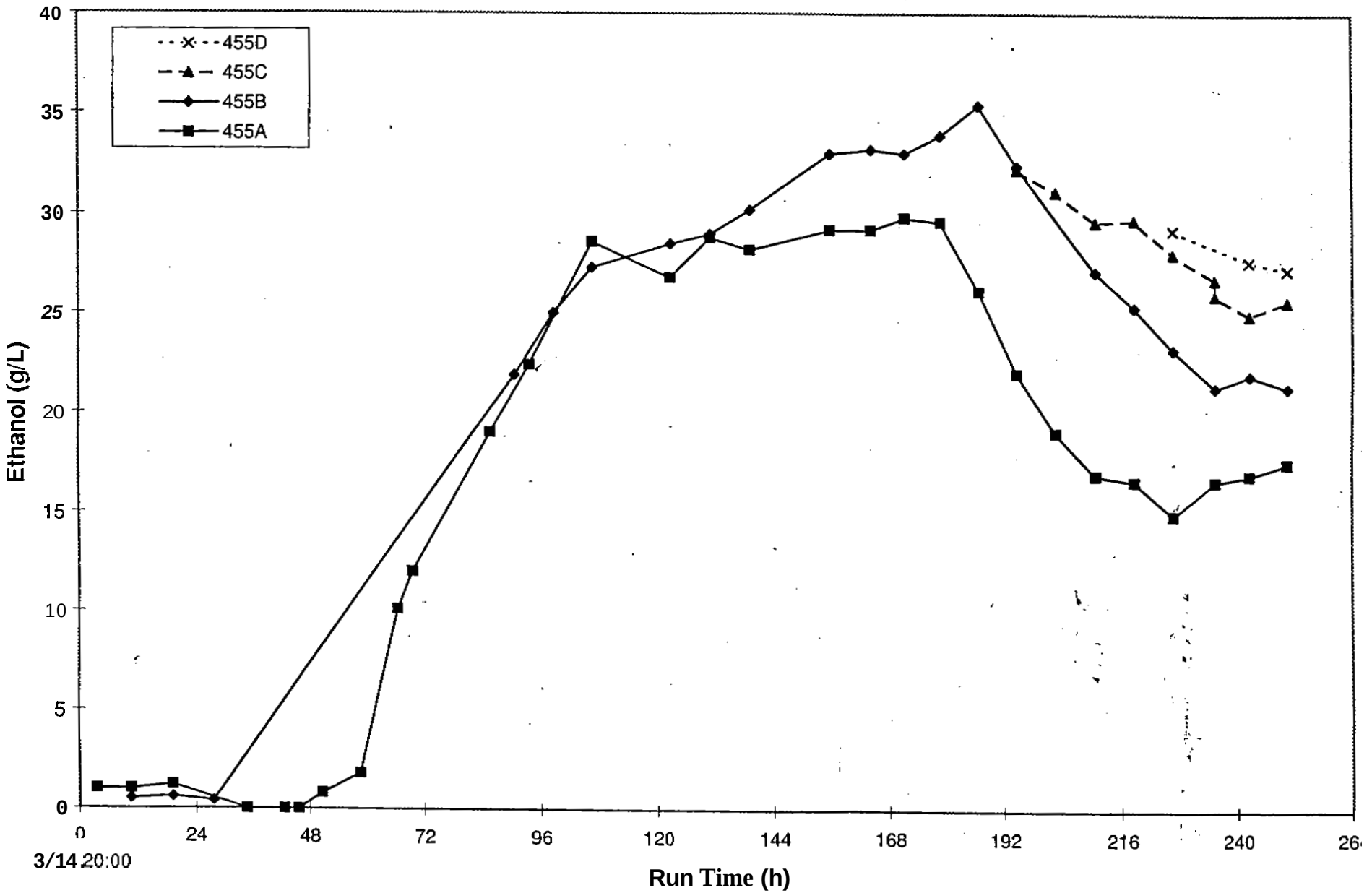


Figure 6. Ethanol in the 9000 L Fermenters



Acid Concentration Data
Figure 3

Date	Time	Run time (h)	Acid Conc. (%)
3/22/95	10:00	4.00	0.38
3/22/95	12:00	6.00	0.54
3/22/95	16:00	10.00	0.55
3/22/95	18:00	12.00	0.47
3/22/95	20:00	14.00	0.4
3/22/95	22:00	16.00	0.54
3/23/95	0:00	18.00	0.41
3/23/95	1:00	19.00	0.5
3/23/95	4:00	22.00	0.47
3/23/95	6:13	24.25	0.82
3/23/95	8:00	26.00	0.58
3/23/95	16:00	**34.00**	0.63
3/23/95	18:00	36.00	0.55
3/23/95	21:00	39.00	0.5
3/24/95	0:00	42.00	0.75
3/24/95	2:00	44.00	0.35
3/24/95	4:00	46.00	0.55
3/24/95	6:00	48.00	0.4
3/24/95	8:00	50.00	0.44

Corn ***Fiber*** Hydrolyzate Composition
Figure 4

Date	Time	Run time (hrs)	HPLC (g/L)											
			Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
3/22/95	9:00	2:30	4.6	9.6	3.3	15.9	0	0	2.5	2.3	1.6	0	0.1	0.9
3/22/95	13:20	6:50	3.1	7.1	2.6	11.8	0	0	1.3	0	1.4	0	0	1.1
3/22/95	17:00	10:30	3.3	6.8	2.4	10.7	0	0	1.6	0	1.3	0	0	0.8
3/22/95	20:30	14:00	4.1	8.2	2.9	12.5	0	0	1.9	0.6	1.3	0	0	0.9
3/22/95	21:00	14:30	3.8	7.6	2.7	12.6	0	0	1.7	0.6	1.2	0	0	0.8
3/23/95	1:00	18:30	3.4	6.8	2.5	12.1	0	0	1.7	1.3	1.3	0	0	0.6
3/23/95	5:00	22:30	3.3	6.6	2.5	11.9	0	0	1.7	0.5	1.2	0	0	0.6
3/23/95	9:00	26:30	3.6	7.5	2.6	11.7	0	0	1.5	1.5	1.1	0	0	0.8
3/23/95	12:00	29:30	3.9	6.3	2.2	10.8	0	0	2	0	1.1	0	0	0.6
3/23/95	18:30	36:00	4.3	6.9	2.5	11.8	0	0	1.9	0	1.1	0	0	0.6
3/23/95	21:00	38:30	4.1	6.7	2.4	11.7	0	0	2	0	1.1	0	0	0.6
3/24/95	1:00	42:30	4.2	7.2	2.7	12.5	0	0	2.1	0	1.2	0	0	0.7
3/24/95	5:00	46:30	4.7	7.8	2.8	12.7	0	0	0.8	0.9	1.1	0	0	0.8

Fermentation Data
Figures 5 and 6

	Date	Time	Run time (h)	HPLC (g/L) Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
t_0=19:30	455A														
3/14/95	3/14/95	23 00	4	14	75	26	82	0	73	51	26	69	1	04	1
	3/15/95	6 00	11	147	86	28	89	0	83	46	21	64	1	04	12
	3/15/95	1435	19	18.2	102	3.2	10.2	0	96	385	247	55	12	03	11
	3/16/95	6 00	35	69	98	34	109	0	0	075	0	17	0	05	24
	3/16/95	1400	43	62	9.9	33	131	0	34	0.8	0	16	0	04	19
	3/16/95	1700	46	11	101	3	123	0	8.2	13	12	22	0	02	15
	3/16/95	2200	51	17	108	29	116	0	98	16	09	21	08	02	11
	3/17/95	6 00	59	18	11.2	29	11.4	0	103	18	11	22	18	02	06
	3/17/95	1400	67	61	118	31	105	0	0	197	21	23	101	0	0
	3/17/95	1700	70	23	10.4	27	92	11	0	18	19	2	12	0	0
	3/18/95	600	86	09	102	27	8.8	0.8	0	18	2	1.8	19	0	0
	3/18/95	14:00	94	0.9	102	2.7	8.8	0.8	0	17	227	16	224	0	0
	3/19/95	6 00	107	07	8.6	22	72	07	0	16	23	13	286	0	0
	3/19/95	2200	123	1.4	8.8	2.3	75	0.8	0	16	21	1.4	266	0	0
	3/20/95	6 15	131	43	79	22	67	0	0	1.4	2.3	07	28.8	0	0
	3/20/95	1430	139	11	79	21	67	0	0	13	23	06	262	0	0
	3/21/95	700	156	42	71	2	61	0	12	13	28	06	292	0	0
	3/21/95	1530	164	14	7.4	21	62	0	0	1.3	2.7	0.8	29.2	0	0
	3/21/95	2230	171	07	7	21	6	0	0	16	24	07	298	0	0
	3/22/95	6 00	179	0	66	19	55	0	0	16	26	09	296	0	0
	3/22/95	1400	187	12	71	22	79	0	0	18	25	13	261	0	0
	3/22/95	22 00	195	22	73	25	89	0	3	1.8	22	15	22	0	0
	3/23/95	6 00	203	21	71	23	9.3	0	36	19	13	16	19	0	0
	3/23/95	1400	211	18	7.3	2.3	10.3	0	4	21	16	17	169	0	0
	3/23/95	22 00	219	22	6.8	19	94	0	43	3.2	05	21	166	0	0
	3/24/95	6 00	227	19	68	1.9	8.8	0	6	46	08	28	149	0	0
	3/24/95	1445	235	21	73	2	59	0	35	9.3	16	45	166	0	0
	3/24/95	22 00	243	16	73	19	45	0	0	10.5	15	54	169	0	0
	3/25/95	6 00	251	1	77	21	35	0	29	11.8	16	63	175	0	0
t_0=19:30	4558														
3/14/95	3/15/95	6 00	11	131	65	25	68	08	0	72	31	101	05	02	1
	3/15/95	1435	19	153	76	27	8	0	0	56	17	82	06	0	08
	3/15195	23 00	28	126	63	26	69	0	0	7.8	41	11.3	04	0.2	1
	3/18195	1400	91	0	9.7	23	83	07	0	19	23	17	219	0	0
	3/18/95	22:00	99	17	8.9	23	76	08	0	17	225	15	25	0	0
	3/19/95	600	107	0.9	8.9	23	75	0.8	0	17	23	15	273	0	0
	3/19/95	22 15	123	3	82	22	69	0	0	17	25	13	265	0	0
	3/20/95	6 15	131	37	79	22	6.8	0	0	17	22	13	29	0	0
	3/20/95	1430	139	0	81	21	68	06	0	16	23	13	302	0	0
	3/21/95	700	156	1.8	73	2	63	0	12	15	25	12	33	0	0
	3/21/95	1530	164	0	76	22	65	0	0	1.5	23	11	332	0	0
	3/21/95	2230	171	0	71	1.9	64	0	0	1.7	1.8	1	33	0	0
	3/22/95	600	179	0	71	21	65	0	0	17	25	1	339	0	0
	322195	1400	187	0	7	2	63	0	0	17	2	09	354	0	0
	3/22/95	2200	195	09	69	21	68	0	0	18	2	11	324	0	0
	323195	1400	211	1	75	25	83	0	0	19	16	14	271	0	0
	3/23/95	22 00	219	1	7.4	2.4	8.3	0	0	24	11	16	253	0	0
	3/24/95	6 00	227	11	7.3	2.4	8.1	0	0	35	11	22	232	0	0
	3/24/95	1445	235	09	79	26	56	0	25	6.5	1.2	3.8	213	0	0
	3/24/95	22 00	243	0	78	26	34	0	23	a4	1.4	51	219	0	0
	3/25/95	6 00	251	0	8	29	17	0	23	98	13	61	213	0	0
t_0=19:30	455c														
3/14/95	3/22/95	2200	195	07	67	2	6	0	0	16	19	1	322	0	0
	3/23/95	6 00	203	07	69	2	67	0	0	1.5	19	11	311	0	0
	3/23/95	1400	211	0	72	22	73	0	0	16	24	12	296	0	0
	3/23/95	22 00	219	06	6.9	22	7	0	0	19	14	14	297	0	0
	3/24/95	6 00	227	06	71	23	7	0	0	256	13	18	28	0	0
	3/24/95	1445	235	0	7	25	48	0	0	35	1.4	26	267	0	0
	3/24/95	1445	235	0	77	27	47	0	0	53	13	34	259	0	0
	3/24/95	22 00	243	0	75	26	33	0	0	6	13	3.9	249	0	0
	3/25/95	6 00	251	0	7.8	3	22	0	0	11	13	47	256	0	0
t_0=19:30	455D														
3/14/95	3/24/95	6 00	227	0	68	22	63	0	0	17	16	16	292	0	0
	3/24/95	22'00	2431	0	73	27	41	0	0	43	14	31	276	0	0
	3/25/95	600	251	0	7	27	3	0	0	4.9	14	36	272	0	0